A Review of Hemoglobin and the Pathogenesis of Cerebral Vasospasm

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We believe that current experimental and clinical evidence can be most satisfactorily interpreted by assuming that oxyhemoglobin is the cause of cerebral vasospasm that follows subarachnoid hemorrhage. We review the pathogenetic mechanisms by which oxyhemoglobin affects cerebral arteries. The relative importance of each of these mechanisms in the genesis of vasospasm, the biochemical pathways of oxyhemoglobin-induced smooth muscle contraction, and the intracellular actions of oxyhemoglobin on smooth muscle and on other cells in arteries are still not definitely established. (Stroke 1991;22:971-982)

Cerebral vasospasm is an important cause of cerebral ischemia and death1-3 following aneurysmal subarachnoid hemorrhage (SAH). Cerebral vasospasm is usually the most frequent serious complication in survivors of SAH,1-3 although recent reports show that with hypervolemic hypertensive therapy and calcium channel antagonists, morbidity and mortality from vasospasm may have become relatively less important.4

Advances have also been made in identifying the spasmogen responsible for vasospasm and in defining how it causes arterial narrowing. Oxyhemoglobin (OxyHb) is probably the principal pathogenetic agent.5-7 We review evidence supporting the theory that OxyHb causes vasospasm. This substance has many mechanisms of action that may be important in vasospasm. These include the release of free radicals8,9; the initiation and propagation of lipid peroxidation9; metabolism to bilirubin, which is another potential spasmogen10-11; the release of vasoactive eicosanoids12 and endothelin13 from the vessel wall; perivascular nerve damage14-15; the inhibition of endothelium-dependent relaxation16; and the induction of structural damage in the arterial wall.15

Attempts to Isolate the Spasmogen

Incubation of blood in vitro results in slow hemolysis of erythrocytes (RBCs) with release of OxyHb into the supernatant fluid starting after 2 days.9,17-23 With time OxyHb is oxidized to methemoglobin,9,17,18,20 but further breakdown of heme to bilirubin occurs only in vivo.6,17,24 The exact mechanisms by which heme groups of hemoglobin (Hb) are broken down into bilirubin are not known, but they probably require enzymes present only in living cells.25 After SAH, RBCs remain fixed in the subarachnoid space for days and they disappear by hemolyzing, similar to the process in vitro.17,26

In 1944, Zucker27 recognized that RBC cytosol was vasoactive although platelets and serum were much more potent vasoconstrictors. Further experiments have documented that RBCs contain a vasoactive substance that is released by hemolysis.18-22,28-37 Humans with clinically significant vasospasm on average have higher temperatures and more often have leukocytosis than patients with ruptured aneurysms without vasospasm.38 In experimental animals, hemogenic meningitis, which may produce these changes, is due to the heme component of RBCs.39,40

Many investigators have assayed the vasoactivity of incubated and aged mixtures of whole blood, blood components, and cerebrospinal fluid (CSF) on dog and cat basilar arteries in vitro.18-20,22,31-33,41 Results are remarkably concordant between experiments and may be summarized as follows. Fresh serum, platelet-rich plasma, and lysed RBCs have significant vasoactivity whereas fresh, intact RBCs are inert. With incubation, serum and platelet-rich plasma become inactive whereas the contractile activity of intact or lysed RBCs persists. Similar experiments were performed in vivo by McIlhany and colleagues,35 who injected vasoactive fractions of hemolyzed RBCs, as collected on Sephadex G-75 gel filtration chromatography, into the cisterna magna of dogs. Vasoconstriction of the basilar artery was observed for 2 days. Peterson et al42 found that RBC lysis was necessary...
for development of vasospasm after the subarachnoid injection of blood into dogs. In summary, during the time of vasospasm in man, the vasoactivity of blood incubated in vitro, when tested on the basilar artery in vitro, resides in RBCs.

The role of RBCs in generating vasospasm has been evaluated further by long-term in vivo studies. The RBCs are the blood component necessary for development of vasospasm in cats and dogs. Mayberg et al developed a piglet model whereby blood components could be selectively applied to the middle cerebral artery (MCA) for 10 days. Whole blood caused a significant reduction in lumen diameter. Selective application of washed RBCs, RBC cytosol, and pure porcine Hb also caused significant vasospasm whereas leukocytes and platelet-rich plasma did not.

The vasospastic substance released by RBCs has been isolated by many groups. The compound has consistently been found to be a peptide with a molecular weight, spectrophotometric absorption pattern, and movement on electrophoresis similar or identical to those of OxyHb. Only Cheung et al found a molecular weight (40,000–45,000 d) that is low for Hb, which has a molecular weight of 64,500 d.

These investigations show that RBCs are essential for the development of vasospasm. Results of studies isolating the spasmogen in RBCs and of long-term in vivo studies have pointed to OxyHb as the prime candidate to cause vasospasm.

**Studies of Cerebrospinal Fluid After Subarachnoid Hemorrhage**

In man, vasospasm has its onset 3 days after SAH, peaks after 6–7 days, and resolves by 14 days. This unusual time course could be explained by the spasmogen responsible for vasospasm exhibiting a similar time course of appearance in the CSF near arteries in spasm. An alternative hypothesis explains the delay in onset by suggesting that arterial narrowing is due to another mechanism such as fibrosis and inflammatory-cell infiltration of the arterial wall or to intimal proliferation. In our opinion, this latter hypothesis is untenable for a variety of reasons. There is no evidence that arterial wall area increases enough during vasospasm to narrow the lumen. Intimal proliferation and arterial wall fibrosis typically develop after vasospasm resolves.

Pathology of the subarachnoid space following human SAH shows that within 24 hours after SAH there is intense polymorphonuclear cell infiltration of the meninges. Phagocytosis and breakdown of RBCs occur by 16–32 hours. Breakdown of RBCs peaks around day 7 but continues for days, with clumps of intact RBCs still enmeshed in the arachnoid for up to 35 days after SAH. At 7 days, the inflammatory reaction subsides and is composed equally of lymphocytes and phagocytes. After 10 days, fibrosis of the subarachnoid space begins. Therefore, during the time of vasospasm, the most prominent process occurring in the subarachnoid space is RBC hemolysis. Clearance of blood injected into the subarachnoid space of animals is rapid, and most RBCs are removed by methods other than hemolysis. This is probably why single injections of liquid blood in experimental animals fail to mimic human vasospasm.

Hemolytic breakdown of RBCs observed microscopically may also be observed by spectrophotometric examination of pigments released into the CSF. Barrows and colleagues used absorption spectrophotometry to examine CSF after SAH; OxyHb appeared 2 hours after SAH. By 4 days postictus bilirubin was present, and over the following week the amount of OxyHb decreased and that of bilirubin increased. In the absence of further bleeding, both pigments disappeared after 2–3 weeks. Bilirubin is present in normal serum and could be observed in the CSF immediately after massive SAH. In cases of SAH methemoglobin was not detected, although it is now believed to be present. Results of these and other studies show that OxyHb is present during vasospasm whereas small molecules and proteins such as albumin, noradrenaline, and serotonin are cleared rapidly from the CSF.

Buckell first investigated the contractile activity of CSF from patients with ruptured aneurysms. Some CSF activity could be accounted for by serotonin, but in half the cases other substances were present that caused contraction. The contractile activity of xanthochromic CSF has been frequently studied. Allen et al suggested that the vasoactivity of xanthochromic CSF was due to serotonin. Other investigators, however, report that drugs that block serotonin, histamine, acetylcholine, norepinephrine, epinephrine, propranolol, and angiotensin II do not prevent hemorrhagic CSF from contracting cerebral arteries in vitro. Calcium channel antagonists have consistently at least partially blocked such contractions. Evidence reviewed below indicates that OxyHb-induced contractions show pharmacological properties similar to those of the agent in xanthochromic CSF. Sasaki et al further delineated the nature of the contractile activity by showing that disulfide bond–reducing agents blocked contractions induced by xanthochromic CSF in dog basilar artery. Disulfide bond reducers are known to block smooth muscle contraction due to prostaglandins (PGs), Hb, and lipid peroxides. Yamamoto and colleagues isolated a spasmogen from xanthochromic CSF that was believed to possibly be endothelin, although the system used to assess vasoactivity was unconventional.

If the vasoactivity in xanthochromic CSF is due to OxyHb, then CSF OxyHb concentration should correlate with severity of vasospasm. This relation has been difficult to confirm, however, partly because lumbar CSF OxyHb concentrations do not accurately reflect concentrations adjacent to spastic arteries. Otta and coworkers found that vasospasm did correlate with the concentration of OxyHb in periarterial hematomas after SAH. An additional problem with assays in vitro is that OxyHb may not be able to...
Hemoglobin as a Vasocostricor

Vasospasm probably begins as smooth muscle contraction, but due to the prolonged and severe nature of the contraction, vasoconstriction becomes temporarily irreversible.848679.975648-78 There may be changes in vessel wall collagen and in the contractile apparatus of smooth muscle that contribute to narrowing and cause stiffness of the arterial wall.848656.8082 Morphological changes in vasospasm and their relation to OxyHb are reviewed below. Studies of the effects of Hb solutions on cerebral arteries both in vitro and in vivo are therefore relevant to theories implicating Hb as a cause of vasospasm.

Studies In Vitro

In vitro, Hb contracts isolated smooth muscle cells and cerebral arteries of several different animal species.851212.2312.34767983104 Fujii and Fujitsu83 reported that OxyHb contracted smooth muscle cells cultured from rat aorta. Ultrastructural changes including vacuolation, cell degeneration, and loss of internal cell structure were noted after 24 hours of exposure. We have used electrophysiology to study smooth muscle cells isolated from rat cerebral arteries.84 A solution containing OxyHb and small amounts of methemoglobin contracted cells and increased calcium-dependent potassium currents. Cells died rapidly after exposure. Pure methemoglobin solution did not cause any of these effects.

Cook et al85 demonstrated that Hb causes slowly developing and long-lasting contraction of dog cerebral arteries, rabbit ear arteries, and rat stomach fundus in vitro. These workers used a solution of Hb containing 20% OxyHb. Asano and associates9121 performed similar experiments on dog basilar artery in vitro and found that both OxyHb and methemoglobin caused dose-dependent contractions, although OxyHb was much more potent. These results are consistent with many others.868791103

Systemic and cerebral arteries have differing pharmacological properties; these arteries also vary in their sensitivity to OxyHb, providing one reason for the predilection of vasospasm for cerebral arteries.8687102

Studies In Vivo

Many studies have investigated the short-term effects of Hb applied to cerebral arteries exposed in situ.208676105113 In 1975, Chokyu105 exposed rat basilar artery in situ and demonstrated a 30% constriction due to the topical application of hemolysate of RBCs and a 19% constriction due to pure OxyHb. Similar to results in vitro, others have shown a similar activity of OxyHb and have generally shown that methemoglobin is much less vasoactive.30107112 Only Fox106 reported that Hb did not contract canine basilar artery exposed in situ, but it is unclear if the Hb solution used contained OxyHb.

Most of the above experiments confirm that OxyHb causes vasoconstriction. Pure methemoglobin is probably inert, but the presence of small amounts of OxyHb will render a solution vasoactive. Wellum et al7988 have expressed concern that OxyHb is not a strong enough vasoconstrictor to cause severe vasospasm. However, if OxyHb is responsible for vasospasm then short-term experiments in vitro would not replicate conditions in vivo, where arteries are exposed to high concentrations of OxyHb for many days. The concentration of OxyHb used in most in vitro and short-term in vivo studies ranges from 10^{-8} to 10^{-2} M, with maximal contraction developing at concentrations equal to and above 10^{-3} M, depending on the preparation.858695919498101 Measurements of Hb concentrations in subarachnoid hematomas76 suggest that adequate amounts of OxyHb are present near vasospastic arteries for long enough to produce maximum contraction.

The issue of duration of exposure to the spasmoconstrictor has been addressed in experimental models of vasospasm where changes in arterial diameter can be assessed over many days. Single intracisternal injections of low doses of OxyHb have been given to small numbers of dogs9114116 and baboons117 with variable results but, unfortunately, single injections do not produce a model that is similar to the human condition, where large amounts of OxyHb are present adjacent to cerebral arteries for days.

Mayberg and colleagues45 developed a piglet model of SAH that kept high concentrations of Hb or various blood components adjacent to the right MCA for 10 days. Pure porcine Hb (roughly 15% as OxyHb) resulted in a significant decrease in lumen area that was equal to that caused by RBC cytosol containing a similar amount of Hb. Although whole blood caused a greater decrease in lumen area than did Hb, these authors suggested that this was because the former was associated with more residual clot-containing Hb at day 10. Therefore, all vasoactivity of the whole blood was accounted for by Hb.

In our laboratory, cynomolgus monkeys had catheters placed along the right MCA and connected to subcutaneous CSF reservoirs.8 In a randomized and controlled study of 40 monkeys, multiple intrathecal injections of OxyHb, methemoglobin, bilirubin, mock CSF, or supernatant fluid from an incubated mixture of autologous blood and mock CSF were given for 6 days. Significant vasospasm of the right MCA, as judged by comparison of angiograms taken at baseline and on day 7, developed in animals injected with OxyHb and supernatant fluid. Pure methemoglobin produced no significant arterial narrowing.

Mechanisms of Oxyhemoglobin-Induced Vasoconstriction

Studies of How Oxyhemoglobin Causes Vasocostricor

Contraction caused by receptor-operated systems usually decreases with time due to tachyphylaxis, desensitization, and/or autoregulation.7879 The time...
course of vasospasm suggests that smooth muscle contraction by a conventional receptor-operated system is not the sole mechanism responsible for arterial narrowing. Most agents suggested to be mediators of vasospasm, such as serotonin, biogenic amines, peptides, and eicosanoids, however, probably act on cell surface receptors.\textsuperscript{45,79,118} Further, antagonists of receptor-operated vasoconstrictors, including atropine, methysergide, cinanserin, ketanserin, phenoxbenzamine, phentolamine, mepramine, chlorpheniramine, propranolol, salbutamol, angiotensin, sarcosine, alanine, theophylline, and quinine, have consistently failed to reverse OxyHb-induced contractions of cerebral arteries in vitro and in vivo.\textsuperscript{31,36,76,85-87,94,95,98,103,107-109,112,119-121}

Drugs reported to relax smooth muscle contracted with OxyHb include papaverine, calcium channel antagonists, and some inhibitors of eicosanoid synthesis.\textsuperscript{85,86,95,108,109,119-121} The latter are discussed in the next section. Although calcium channel antagonists partly reverse OxyHb-induced contraction of cerebral vessels in vitro, these drugs do not dilate monkey\textsuperscript{122} or human cerebral arteries that are vasospastic. Kajikawa et al\textsuperscript{108,109} found that after exposing rat basilar arteries in situ to barium chloride for 3 hours, papaverine did not dilate them, providing evidence that the duration of contraction bears upon the ability of pharmacological antagonists to relax smooth muscle.

OxyHb acutely increases the intracellular concentration of inositol phosphates, which are second messengers involved in smooth muscle contraction.\textsuperscript{125} There are few other reports investigating the intracellular mechanism of action of OxyHb, which is obviously an important question.

**Eicosanoids**

Eicosanoids are products of enzymatic metabolism of arachidonic acid and include PGs, thromboxanes, and leukotrienes.\textsuperscript{126}

After SAH in monkeys and dogs, vasospastic vessels synthesize less PG\textsubscript{1} and more vasoconstricting PGE\textsubscript{2}.\textsuperscript{126-130} Decreased vasodilator influence from PG\textsubscript{1}, combined with endothelial damage with adherence of platelets and release of vasoconstricting PGs and thromboxane A\textsubscript{2}, could contribute to vasospasm.\textsuperscript{3,12,130} The effects of OxyHb on vessel wall eicosanoid synthesis in vivo have been investigated by Tokoro.\textsuperscript{129} He reported that injection of OxyHb into dog cisterna magna decreased PGI\textsubscript{2} levels in the CSF containing elevated levels of PGF\textsubscript{2}α and possibly other PGs.\textsuperscript{126,131-133} Dog basilar artery removed 6 days after SAH synthesizes more PGE\textsubscript{2} than normal.\textsuperscript{127,128} Experiments in vitro using bioassay systems to measure eicosanoids show that OxyHb releases vasoactive PGs from several types of blood vessels,\textsuperscript{12,124,135} potentially accounting for the changes seen after SAH. Todo\textsuperscript{135} concluded that OxyHb-induced contraction was dependent on a mechanism related to the endothelium but not involving endothelium-derived relaxing factor (EDRF). He suggested that PGs released from endothelium by OxyHb were important in these contractions.

The effects of inhibitors of PG and thromboxane synthesis on experimental and human vasospasm have been equivocal.\textsuperscript{92,135-139} Studies of OxyHb-induced dog basilar artery contraction in vitro show that indomethacin and aspirin, inhibitors of cyclooxygenase, have no effect on\textsuperscript{86,90,103} or only partially prevent\textsuperscript{12,85,87,94,119} such contractions. Thromboxane synthetase inhibitors were also ineffective in preventing OxyHb from contracting dog\textsuperscript{102} and guinea pig\textsuperscript{140} basilar artery.

Thus, although OxyHb affects vessel wall eicosanoid production and could account for the alteration in CSF levels of these substances after SAH, inhibitors of synthesis of PGs and thromboxanes do not prevent vasospasm. If OxyHb acts at multiple sites in the vessel wall (e.g., direct action on smooth muscle cells by the release of free radicals and the production of lipid peroxides, combined with the release of eicosanoids, EDRF, and endothelin from the endothelium), then antagonizing only one of these systems might not completely reverse the contraction. Furthermore, other factors, such as leukotrienes, which are also potent vasoactive agents and the concentrations of which increase after SAH, have not been thoroughly investigated.\textsuperscript{141-143}

**Free Radicals**

OxyHb spontaneously autoxidizes to methemoglobin, releasing superoxide anion radical (O\textsubscript{2}·\textsuperscript{-}).\textsuperscript{8,144} Spectrophotometric studies show that after SAH, hemolyzing subarachnoid RBCs release OxyHb, which autoxidizes to methemoglobin, potentially releasing O\textsubscript{2}·\textsuperscript{-} into the subarachnoid space.\textsuperscript{55-59} In conjunction with the iron in Hb, O\textsubscript{2}·\textsuperscript{-} has been postulated to initiate and propagate lipid peroxidation by the Haber-Weiss reaction and Fenton chemistry.\textsuperscript{9,21} Lipid peroxides are known to cause vasoconstriction and structural damage to cerebral arteries both in vitro\textsuperscript{145,146} and in vivo.\textsuperscript{147,148}

There is additional evidence for a role of lipid peroxidation, and therefore OxyHb, in the genesis of vasospasm. Vasoreactivity of blood incubated in vitro correlates with its concentration of OxyHb and with its content of lipid peroxides, represented by thiobarbituric acid–reactive substances.\textsuperscript{9,21,147} Concentrations of lipid peroxides in the CSF of patients with SAH correlate with vasospasm.\textsuperscript{9,21,147,149,150} An inhibitor of iron-dependent lipid peroxidation, U74006F, significantly diminished vasospasm in a primate model of SAH.\textsuperscript{151} Despite questions about the exact mechanism of initiation of lipid peroxidation,\textsuperscript{152} detection of 5-hydroxyeicosatetraenoic acid in CSF after SAH shows that lipid peroxidation occurs.\textsuperscript{153}

Although OxyHb probably does propagate lipid peroxidation after SAH, it has not been shown in vivo that the process is essential for the development of
vasospasm. This would require evidence that inhibitors of lipid peroxidation prevent vasospasm\textsuperscript{151} and evidence that lipid peroxidation precedes the development of vasospasm since the arterial wall damage that accompanies vasospasm would produce lipid peroxides.\textsuperscript{152} Furthermore, since vasospasm correlates best with large volumes of subarachnoid blood,\textsuperscript{5,154} the concentrations of lipid peroxides in CSF would be elevated in association with vasospasm whether or not their production is related to vasospasm.

Effects of free radical-scavenging enzymes on the vasoactivity of OxyHb on cerebral arteries in vitro have been reported.\textsuperscript{36,96,99,91,94,109} Kamiyama and coworkers\textsuperscript{110} found that superoxide dismutase, catalase, and 1,4-diazabicyclo[2.2.2]octane were effective inhibitors of OxyHb-induced contraction of cat basilar artery exposed in situ. Fujita et al\textsuperscript{89} found superoxide dismutase to be ineffective at antagonizing OxyHb-induced contraction of dog basilar artery in vitro. The effects of catalase alone have also been inconsistent.\textsuperscript{36,91,94}

Electrophysiological studies of isolated rat cerebral arterial smooth muscle cells have shown OxyHb to activate calcium-dependent potassium currents and cause cell death.\textsuperscript{84} Catalase protected cells from OxyHb, whereas superoxide dismutase did not. Xanthine and xanthine oxidase did not cause electrophysiological changes in cells, whereas the generation of hydroxyl radical in the bathing solution was damaging. The findings implicate the hydroxyl free radical rather than other oxygen free radicals although it may be difficult to define the actual free radicals involved based on such scavenger experiments.\textsuperscript{152}

In summary, superoxide dismutase and catalase alone are poor antagonists of OxyHb-induced cerebral artery contraction. There are several possible reasons for this. Free radical mechanisms may not be important or they may not be the sole pathway in the genesis of contraction by OxyHb. Superoxide dismutase and catalase may not be able to penetrate arterial walls to get to the locations where damaging free radical reactions occur.\textsuperscript{152} Activity of both superoxide dismutase and a hydrogen peroxide scavenger may be necessary to prevent the genesis of oxygen-derived free radicals since superoxide dismutase produces hydrogen peroxide, which can form hydroxyl radical in the presence of iron or ferrous proteins.\textsuperscript{155,156} Catalase or glutathione peroxidase, if present, would catabolize hydrogen peroxide, preventing this reaction. Hydrogen peroxide\textsuperscript{157} and iron ions\textsuperscript{158} can also inhibit superoxide dismutase.

**Endothelium-Dependent Relaxation**

Furchgott and Zawadzki\textsuperscript{159} showed that the vasodilatory action of acetylcholine on rabbit aorta was mediated by release of an intermediate substance from endothelial cells. This substance has been termed EDRF. Other vasodilators relax vascular smooth muscle through this endothelium-dependent mechanism, which has been subject to recent review.\textsuperscript{160}

After SAH in dogs, vasospastic basilar arteries contracted with PGF\textsubscript{2}\textalpha{} and uridine 5'-triphosphate exhibit impaired relaxation to arginine vasopressin and thrombin.\textsuperscript{161,162} Endothelium-dependent relaxation is also inhibited after SAH in rabbits\textsuperscript{163,164} and monkeys.\textsuperscript{165} OxyHb and other ferrous hemoproteins but not methemoglobin or ferric hemoproteins are well-known inhibitors of endothelium-dependent relaxation in a number of vascular preparations in vitro.\textsuperscript{15,95,104,121,140,160,166-173} Whether or not endothelium-dependent relaxation occurs correlates with levels of cyclic guanosine monophosphate in the arterial wall.\textsuperscript{166}

A study in vivo of endothelium-dependent relaxation and Hb was reported by Byrne et al.\textsuperscript{113} In pigs, intracisternal injection of OxyHb caused acute vasoconstriction of intrathecal arteries. Intracarotid infusion of acetylcholine caused vasodilation before the intracisternal injection of OxyHb and vasoconstriction afterwards.

Kanamaru et al\textsuperscript{120} found that xanthochromic CSF from SAH patients could inhibit A23187-induced endothelium-dependent relaxation in dog basilar artery in vitro. The levels of OxyHb in CSF correlated with the degree of inhibition of endothelium-dependent relaxation.

The ability of OxyHb to inhibit endothelium-dependent relaxation suggests that OxyHb is responsible for this event after SAH. How OxyHb prevents endothelium-dependent relaxation has been investigated. Both SAH and OxyHb damage arterial endothelium, possibly decreasing EDRF synthesis by endothelial cells.\textsuperscript{154,148,174} Kim et al,\textsuperscript{161} however, used a bioassay system for EDRF to show that EDRF secretion by vasospastic dog basilar artery was not impaired. EDRF may be nitric oxide,\textsuperscript{149} and Hb binds nitric oxide with an affinity 1,500 times higher than its affinity for oxygen.\textsuperscript{175} Thus Hb, which presumably does not enter cells, could prevent EDRF, if it is nitric oxide, from entering smooth muscle cells and producing its effects.

The effects of OxyHb on EDRF have been assessed in vitro using more physiological systems that isolate the intraluminal and extraluminal aspects of the artery. Although OxyHb applied extraluminally to dog and rabbit cerebral arteries has been found to have little effect on endothelium-dependent relaxation,\textsuperscript{170,171} other researchers reported the inhibition of endothelium-derived responses after exposing extraluminal surfaces of cerebral arteries to OxyHb.\textsuperscript{104,172}

The effects of Hb on endothelium are further complicated by observations that acetylcholine causes transient hyperpolarization and relaxation of rat aorta and guinea pig basilar artery precontracted by noradrenaline.\textsuperscript{170} Hb prevents relaxation but doesn’t alter hyperpolarization. It has been hypothesized that acetylcholine releases EDRF and endothelium-derived hyperpolarizing factor from endothelial cells.
In addition to EDRF, endothelium-derived hyperpolarizing factor, and eicosanoids, endothelial cells synthesize a potent vasoconstrictor peptide, endothelin. Vasospasm is associated with increased CSF levels of endothelin. Several early experiments found that bilirubin had no effect on cerebral artery diameters in vitro. In these studies, bilirubin was applied to arteries for minutes only. Duff and colleagues reported that bilirubin solutions caused progressive vasoconstriction of cat and baboon basilar arteries. Electron microscopy of the arteries showed swelling of endothelial cells, degeneration of axons and varicosities in the adventitia, and extensive vacuolation of smooth muscle and endothelial cells. These results were supported by investigations by Miao and Lee that demonstrated the vasoconstrictive effects of bilirubin on cerebral arteries.

Intraarterial injection of bilirubin for 6 days was performed in our laboratory as part of a randomized trial to study the role of various blood components in the etiology of vasospasm in monkeys. Bilirubin caused a nonsignificant 13% decrease in right MCA diameters. Ultrastructural examination of these arteries did show some pathological changes.

Although the time course of appearance of bilirubin in the CSF after SAH is similar to that of vasospasm, there are several reasons to doubt that bilirubin is an important spasmogen. Bilirubin contaminates CSF in other diseases such as neonatal and obstructive jaundice. Focal neurological deficits, which might be related to vasospasm, have rarely been noted in these conditions. Wahlgren and Bergstrom found concentrations of bilirubin in the CSF of patients with obstructive jaundice that caused vasospasm in the study of Duff et al. Bilirubin, especially when bound to albumin, possesses antioxidant activity and inhibits lipid peroxidation. Furthermore, in the subarachnoid space bilirubin is produced by an enzyme system with limited capacity, which may result in bilirubin concentrations that are too low to induce significant arterial narrowing after SAH. Findings that intrathecal injections of OxyHb cause vasospasm whereas injections of methemoglobin do not also cast doubt on the bilirubin theory since both types of Hb produce bilirubin in the subarachnoid space.

### Neurogenic Effects

Cerebral arteries receive adrenergic, cholinergic, and peptidergic innervation and possess receptors for neurotransmitters such as serotonin, α- and β-adrenergic drugs, dopamine, and histamine although the precise role of these nerves and receptors in the regulation of cerebrovascular tone is unknown.

Adventitial nerve endings in cerebral arteries degenerate after experimental SAH, and this is accompanied by a transient loss of catecholamine fluorescence around cerebral arteries. Disappearance of perivascular nerves after SAH, however, did not correlate with vasospasm in rats and primates. Sympathectomy can have no effect on or it can alleviate vasospasm in experimental animals and humans. Drugs that block α-adrenergic, β-adrenergic, and muscarinic receptors have little effect on cerebral vasospasm induced by whole blood and on vasospasm due to OxyHb.

Despite contradictory evidence regarding the significance of changes in cerebrovascular innervation in relation to vasospasm, SAH clearly does damage these nerves and OxyHb may be responsible for this degeneration. Inhibition of vasodilator nerves and potentiation of vasoconstricting nerves by OxyHb may create a favorable setting for the development of arterial narrowing after SAH. Few studies in vivo have been reported and obviously, results might be different when arteries are in situ with anatomically intact innervation. The effects of OxyHb on peptidergic innervation of cerebral arteries have not been investigated in detail.

### Synergistic and Other Effects

OxyHb accentuates the contractile effects of hypoxia, serotonin, potassium, and fibrin degradation products on cerebral arteries in vitro. Recent investigations, however, have suggested that Hb, and presumably OxyHb, is a sufficient cause of vasospasm. White believed that PGs were probably important in vasospasm and that serotonin, plasmin, and antithrombin III were possibly involved. Other major contenders for the cause of vasospasm include bilirubin, endothelin, and lipid peroxides. Since OxyHb is metabolized to bilirubin, it is a potent propagator of lipid peroxidation, and augments the release of eicosanoids and endothelin from arteries, consideration of any one of these substances as the sole cause of vasospasm may be a moot point. Clearly, OxyHb, while potentially the only agent necessary to precipitate vasospasm, acts by diverse mechanisms to cause arterial narrowing.
Hemoglobin and Pathological Changes in Cerebral Arteries

Most investigators agree that vasospasm is, at least initially, due to smooth muscle contraction.\textsuperscript{45,47–49,174} Vasospastic arteries, however, are abnormal pharmacologically\textsuperscript{2,47,82,200,201} and ultrastructurally,\textsuperscript{2,47–49,174} indicating that processes occur during vasospasm that are not simple muscular contraction. Regardless of the significance of these changes, if OxyHb causes vasospasm, then it should produce the particular arterial wall changes that have been observed in association with vasospasm.

Okada et al\textsuperscript{15} injected OxyHb into feline prepontine cistern, causing myonecrosis, transformation of nerve endings, invasion of myointimal cells into the tunica intima, changes in endothelial cell basement membrane, and detachment of endothelial cells after 24 hours. Kajikawa and colleagues\textsuperscript{108,109} applied OxyHb to exposed rat basilar arteries for 3 hours. Vasospasm resulted, and at electron microscopy endothelial cell craters, blebs, and vacuoles were seen. Smooth muscle cells developed vacuoles, nuclear pyknosis, and mitochondrial degeneration. OxyHb caused vacuolation and degeneration of cultured rat aortic smooth muscle cells, whereas serotonin- and norepinephrine-exposed cells were normal.\textsuperscript{83}

A spectrum of pathological changes have also been produced by the exposure of cerebral arteries in vivo to OxyHb.\textsuperscript{6,45} Therefore, OxyHb can produce morphological changes in cerebral arteries that are indistinguishable from those due to SAH.

Summary

There is good evidence that the release of OxyHb from lysing subarachnoid RBCs is a key mechanism responsible for vasospasm. OxyHb is present in high concentrations in CSF during the time of vasospasm. RBCs are the component of blood necessary for vasospasm to develop, and the most vasoactive substance within RBCs is OxyHb. Although it is not as potent a constrictor as some other agents, OxyHb can act by a variety of pathways over a prolonged period to produce arterial narrowing and ultrastructural damage to arteries that are akin to those occurring after SAH. While OxyHb may be the sole initiator of vasospasm, the pathogenesis of action of OxyHb is probably multifactorial, involving direct effects on smooth muscle, release of vasoactive eicosanoids and endothelin from the arterial wall, inhibition of endothelium-dependent relaxation, production of bilirubin and lipid peroxides, and damage to perivascular nerves.

The relative contributions of known mechanisms of action of OxyHb to the pathogenesis of vasospasm are not worked out. How OxyHb causes smooth muscle contraction and the biochemical derangements that it produces in smooth muscle are also key unanswered questions.

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